EHA EUROPEAN HEMATOLOGY ASSOCIATION

Amendment #1 to EHA Research Grant Funding Agreement

This is the first Amendment ("**Amendment #1**") to the EHA Research Grant Funding Agreement, dated 1st of April 2023 (the "**Agreement**") between the **European Hematology Association** ("**EHA**") and [redacted] ("**Grantee**") and **Charles University** ("**Grantee Host Institute**") (collectively, the "**Parties**").

WHEREAS, the Parties have entered into the Agreement to fund the project as described in the Agreement,

WHEREAS, EHA agreed to make a grant to the Grantee Host Institute to fund the project,

WHEREAS, the Parties wish to amend such Agreement;

NOW, THEREFORE, in consideration of the mutual undertakings and agreements hereinafter set forth, the Parties agree to amend the Agreement as follows:

1. Parties agree to extend the terms of the Agreement for a further twelve (12) months starting from 1/4/2024 and ending 31/3/2025:
   a. The grantee will undertake further research as set out in the proposal (Annex 1).
   b. An additional final project report will be shared with EHA at the end of the extension.
   c. A grant of 37 205EUR will be paid to the bank account of the Grantee Host Institution on or about 1/4/2024, as supported by the provided financial overview (Annex 2).

2. Parties agree that the terms and conditions as set out under this Amendment replace and supersede any other agreement or agreements, oral or written, that the parties may have with respect to the subject matters covered by this Amendment.

IN WITNESS WHEREOF, the Parties hereto have executed this Amendment #1 to be effective, valid and binding upon the Parties as of 1/4/2024 (the "**Amendment Effective Date**").

EHA
Signed by: [redacted]

Name: [redacted]

Title: Managing Director of EHA

Date: 4 April 2024

Grantee
Signed by: [redacted]

Name: [redacted]

Title: [redacted]

Date: 4 April 2024

Grantee Host Institute
Signed by: [redacted]

Name: [redacted]

Title:

Date: 08-04-2024

[redacted]

UNIVERZITA KARLOVA
First Faculty of Medicine
BIOCEV
Prumyslova 595, 252 50 Vestec, Czech Republic
[redacted]

In Vestec 2.2.2024

To: European Hematology Association

Supplement to EHA Ukraine Bridge Funding renewal application - [redacted]

A brief overview of the extension of the project proposal and aims to be achieved in the second year.

During second year, [redacted] would primarily continue to work on two interconnected projects. The first project is focused on studies of circulating tumor DNA in lymphomas and the second one on evaluation of functional effect of lymphoma-associated mutations *in vitro*.

It has been shown that tumor cell-derived cell free DNA (circulating tumor DNA, ctDNA) could be detected in various non-cellular body compartments and/or fluids and could be utilized for disease monitoring and cancer-associated mutations detection. We have recently developed our own panel including 540 genes frequently mutated in various types of lymphomas (to be used in Cancer Personalized Profiling by deep Sequencing, or CAPP-Seq, approach) and successfully tested its performance and use for ctDNA detection. In collaboration with five hematology centers in the Czech Republic, we are collecting plasma samples for ctDNA evaluation in relation to prognostic stratification and treatment response evaluation. We would further focus to provide additional evidence for diagnostic utilization of ctDNA detection in specific clinical situations as are intraocular lymphomas (ctDNA detection in vitreous samples obtained within vitrectomy in patients with suspected intraocular lymphomas), CNS lymphomas (cerebrospinal fluids collected at the time of diagnostics and/or therapeutical intervention), or rare cases of T cell lymphomas. All these samples are already being collected and are prepared for ctDNA analysis and testing. [redacted] classification. We aim a publication related to our first ctDNA-based studies to be submitted this year.

Connected to the detection of lymphoma-associated mutations from ctDNA, we are very skilled in generation of specific modified lymphoma cell lines to genetically introduce and evaluate the functional consequences of these mutations. As a part of our long-term interest, we are investigating functional consequences of CD79B mutations on oncogenic B-cell receptor signaling initiation and transmission. In this regard, [redacted] just recently published a review article summarizing the role of CD79A/B molecules in normal as well as malignant B cells. [redacted] will use his gained skills in cell culture and gene editing methodologies to finalize functional testing related to CD79A/B mutations. We aim this project (including [redacted] contribution) to be finalized and a publication to be submitted this year.

Additionally, [redacted] developed and established in our laboratory a new system to expand normal human B lymphocytes last year. He engineered feeder cells (3T3 and L929 murine fibroblasts) to express B lymphocytes survival-promoting and activating molecules (IL4 and CD40L) to support the expansion of human B lymphocytes. He will finalize evaluation of the system for use, e.g., in inhibitors testing (comparing their effect on normal vs. malignant B cells), or for general B cell biology questions. We aim to summarize these results in a short report for submission at the end of the grant extension period.

For a collaborative project, [redacted] also developed an approach for targeted knock out / knock in (KO/KI) at genomic locus of *MAGEA1* gene. Two variants of KI were prepared as reporters of *MAGEA1* expression: KI of GFP to be expressed instead of MAGEA1 and KI of GFP to be expressed from the MAGEA1 locus at the same time together with MAGEA1 protein. The approach is generally working, and the desired modified cell lines will be produced within next months.

The last but not least aim for the extension of the project is to initialize *in vitro* testing of biological effect of nanoparticles, including rare-earth-based orthovanadate nanoparticles, TiO_{2-x} nanoparticles with different $Ti^{3+}(Ti^{2+})/Ti^{4+}$ ratios, and dextran-graft-polyacrylamide/zinc oxide nanosystems to screen their anti-tumor activity against lymphoma cell lines and to uncover molecular mechanisms underlying their eventual anti-cancer effects.

[redacted]
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Annex 2

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[redacted]

In Vestec 2.2.2024

To: European Hematology Association

Supplement to EHA Ukraine Bridge Funding renewal application - [redacted]

A financial overview of anticipated costs for the additional year.

[redacted]

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